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OCCURRENCE OF VIRUSES IN TREATED DRINKING WATER IN THE UNITED STATES

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ABSTRACT

Health concerns regarding waterborne transmission of enteric viruses began to develop around 1940 in the United States (U.S.) with the isolation of poliovirus from human feces and sewage. The implication of these isolations for the transmission of viral disease through contaminated drinking water stimulated research on methodology for virus detection, recovery and assessment from water. Although virus methods research is still an important area of study, relatively sensitive procedures became available during the past decade for recovering many enteric virus types from large-volume samples of drinking water. Controversy surrounded many of the early reported isolations of viruses from treated drinking water using these procedures due to the suspicion of laboratory contamination. The occurrence of viruses in drinking water treated by currently accepted procedures has still not been proven by the U.S. experience although the likelihood may be gaining support. However, a virus survey of 54 water supplies and extensive studies of two water systems by the U.S. Environmental Protection Agency did not demonstrate viral contamination of treated water derived from surface sources.

KEYWORDS

Water, viruses, water treatment, viruses and drinking water, virus survey.

INTRODUCTION

In 1913, the viral agent of poliomyelitis became the first enteric virus to be detected in human stools in the United States (U.S.) (Sawyer, 1915). However, public health research interest in this finding did not develop until the early 1940s. During this period additional studies using intact rhesus monkeys as the host system demonstrated viable poliovirus in raw domestic sewage (Paul and Trask, 1941). With the very vivid historical evidence that sewage contamination of drinking water had resulted in major waterborne outbreaks of enteric bacterial diseases, it seemed reasonable that contaminated water was also a likely major transmission route for poliomyelitis. Although this hypothesis was not accepted by many in the public health field (Maxcy, 1943), it did provoke considerable investigation in the 1940s into the environmental stability of viruses and the effect of sewage and water treatment processes on their removal and inactivation.

Virus detection and quantitation was significantly limited by the assay system available at that time which was the production of disease in inoculated monkeys, or mice with the subsequent development of mouse-adapted virus strains.

Epidemiological evidence has been insufficient to convince most interested persons that poliomyelitis was a drinking water problem in the U.S. Continued waterborne virus health concerns have rather been stimulated by convincing epidemiological evidence for waterborne hepatitis A outbreaks (Mosley, 1967) and the circumstantial evidence that resulted from virus occurrence studies using the newly developed cell culture detection system. Some 100 viruses that produce a wide variety of disease symptoms were isolated from human stool specimens (unfortunately, hepatitis A virus was not one of these) in a relatively short period of time around mid-century. It seemed likely that a water health problem could be associated with these enteric viruses.

Environmental virology research was begun in a U.S. government laboratory in Cincinnati in 1950 with a study to determine the chlorine sensitivity of coxsackievirus A2. The finding that this virus was significantly more resistant to free chlorine inactivation than coliform indicator bacteria provoked increased interest in the potential for drinking water virus transmission. Subsequent studies by many investigators using cell culture assay systems and crude virus concentration procedures detected human enteric viruses in raw and treated sewage and wastewater-impacted surface waters. The reported isolation of viruses from the drinking water of Paris, France (Coin et al., 1964) further stimulated interest in water virology in the U.S.

REPORTED VIRUS ISOLATION FROM DRINKING WATER IN U.S.

The first reported finding of viruses in treated drinking water in the U.S. occurred in 1970. The unpublished, though highly publicized, findings of a government research team was the isolation of echovirus, poliovirus and reovirus from 7 of 64 samples of conventionally-treated (flocculated, sedimented, filtered and disinfected) water from the supplies of two Massachusetts communities (McDermott, 1974). These findings were controversial due to the suspicion of laboratory error. A subsequent, more tightly controlled study of 10 treated water supplies in three geographical locations was conducted by the U.S. Environmental Protection Agency (USEPA). One of 80 water samples yielded a poliovirus isolate (Clarke et al., 1975). Again, the positive finding was suspect due to the circumstances of the isolation. The water was of excellent quality having no detectable turbidity or coliform bacteria and a free chlorine residual of 1.0 mg/L. In addition, the same virus type was isolated in the laboratory a few days later from a negative control cell culture.

In 1975, a rather extensive virus monitoring program was initiated for a conventionally-treated water supply in Virginia. Over several months, 120 water samples of 380 liters each were concentrated and assayed for viruses. Five of these samples yielded poliovirus isolations. Again, the nature of these isolations provoked concern for their authenticity (Akin and Jakubowski, 1976). However, clear evidence of laboratory error was not found after extensive investigation (Metcalf, 1978; Hoehn and Randall, 1981). In Metcalf's (1978) report of this work, mention is made of an additional drinking water isolation of poliovirus from three samples of treated water from an unnamed major U.S. city. Turbidity, coliform and chlorine residual were reported to be of acceptable values.

In addition to these reported isolations of virus from municipal water supplies during routine monitoring programs, viruses have been isolated on a few occasions in association with waterborne outbreaks of disease. Mack et al. (1972) isolated

poliovirus from a restaurant's untreated groundwater supply that had been implicated in cases of gastroenteritis. Poliovirus was not believed to be the etiological agent, but its isolation indicated the viral contamination of the water supply. Wellings et al. (1975) recovered echovirus 22/23 from a chlorinated groundwater supply that was implicated in an extended outbreak of gastroenteritis in a migrant worker camp. The same virus type was isolated from 6 of 23 stools examined which suggested a causal association.

In an effort to confirm the water source of viral disease outbreaks, the USEPA supported a viral study of outbreak-associated drinking water supplies (Riggs and Spath, 1983). In this study, coxsackievirus was isolated from a 380-liter concentrated sample of drinking water associated with a gastroenteritis outbreak on an Indian reservation. The chlorinated (supposed) and filtered groundwater supply was obtained from a wet well near a creek. Results of immune electron microscopy of stool and sera specimens implicated a Norwalk-like virus as the etiological agent. The coxsackievirus isolation clearly indicated the viral contamination of the water. Subsequent investigation showed that the chlorinator had failed during the outbreak period. On two other occasions, viruses were isolated from untreated waters that were the source of drinking water recently associated with cases of gastroenteritis.

In June, 1980, the largest U.S. outbreak of waterborne disease in recent times occurred in a small Texas community. Eighty-one percent (8,000 persons) of the residents were believed to have had gastroenteritis as a result of consuming contaminated groundwater. An excess number of cases of hepatitis A was also associated with ingestion of the water. Coxsackieviruses B2 and B3 were isolated from sewage samples, untreated water from two of the wells serving the community and from one tap water sample. Coxsackievirus may have had some etiological role in the outbreak. However it is more likely that a Norwalk-like virus was responsible for most of the gastroenteritis cases. There was also weak evidence of hepatitis A viral antigen in one of the wells as determined by radioimmunoassay (Hejkal et al., 1982).

TABLE 1 REPORTED ISOLATIONS OF VIRUSES FROM DRINKING WATER IN THE UNITED STATES

Virus Type	Sample Volume (L)	No. Pos. No. Taken	Water Treatment	Location Date	Reference
Polio, Echo, Reo	19	7/64	Complete*	Mass. 1969-71	McDermott 1974
Polio	19	1/1	Untreated	Michigan 1970	Mack et al. 1972
Polio	19 & 950	1/7	Complete	Mass. 1972-73	Clarke et al. 1975
Echo	380	1/10	Chlorinated	Florida 1975	Wellings et al. 1975
Polio	380	5/129	Complete	Virginia 1975-76	Hoehn & Clifford 1981
Polio	363-442	3/?	Complete	Southern State, 197?	Metcalf 1978
Coxsackie	380	1/1	Filtered†	California 1980	Riggs & Spath 1983
Coxsackie	736-1848	1/8	Chlorinated	Texas 1980	Hejkal et al. 1982

*Complete treatment includes flocculation, sedimentation, filtration and disinfection

†Chlorination was also practiced but not during the outbreak period

A summary of eight reports of virus isolations from drinking water supplies in the U.S. is given in Table 1. Poliovirus was the sole virus type isolated in most of the routine monitoring studies. Only Coxsackie and echo viruses were isolated from treated supplies associated with waterborne outbreaks of disease. The significance of this observation is unclear and may only be an artifact of the small number of positive findings. It is interesting to note, however, that the poliovirus isolations were primarily associated with the supplies that practiced complete treatment and were the more controversial findings.

VIRUS SURVEY OF DRINKING WATER SUPPLIES

The availability of procedures and equipment for concentrating and recovering viruses from large volumes of water (Wallis et al., 1972; Hill et al., 1974) plus the development and evaluation of a standardized recovery method (Standard Methods, 1976; Hill et al., 1976) provided the essential technical prerequisites for a virus survey of drinking water. The USEPA initiated such a survey in 1974 using the recovery procedure published in the 14th edition of Standard Methods (1976). This method provided options in the choice of a virus adsorbent. Millipore 293 mm disc nitrocellulose filters were chosen as the virus adsorbent and glycine buffer pH 11.5 was used as the eluant. Reconcentration of the 1 liter eluate was by readsorption to 47 mm fiberglass-asbestos filters at low pH (3.5) and elution with high pH (11.5) glycine. The final volume was 20 to 80 ml.

Shortly after the survey began, studies in our laboratory indicated that the fiberglass filter tube had advantages over the other optional adsorbents (Jakubowski et al., 1975). It was used throughout the remainder of the study and the sample volume was increased from 380 to 1,900 liters. The only subsequent methodology change of significance was made late in the study and involved modification of the elution and reconcentration step. The organic flocculation procedure of Katzenelson et al., (1976), that was incorporated into the Tentative Standard Method of the 15th edition of Standard Methods (1981), was adopted since it had been found to increase sensitivity over the glycine elution procedure, especially for those enteric viruses adversely affected by the highly alkaline conditions, i.e., adeno and reo viruses.

A major concern of the study was the prevention of sample contamination. The suspicion of such an occurrence had compromised the results of earlier reported virus isolations from water. Since the goal of the virus recovery and assay procedures was to maximize the detection of a single waterborne virion, the opportunity for sample contamination had to be kept at an absolute minimum even though little experience and guidance was available to define the control conditions that would be required. Our controls included aseptic technique and as "closed" a system as possible in the field operation. The laboratory phase of sample processing was conducted in a unique isolation laboratory with workers wearing sugical masks and gowns. Only drinking water samples were freeze stored, processed and assayed in the isolation laboratory.

A quantal CPE assay method was chosen over the quantitative plaque assay procedure because it was believed to be more sensitive for detecting the range of enteric viruses that may be recovered. Initially, the 20 to 80 ml of sample concentrates were inoculated in equal volumes onto monolayers of primary monkey kidney (PMK) cells, primary human embryonic kidney (HEK) cells, and continuous cell lines of monkey kidney (Vero) and porcine kidney. As with the concentration methodology, the viral assay system also evolved as more data became available. The porcine kidney cells were replaced with continuous African green monkey kidney cells (BGM) with the reported greater sensitivity of this cell line for recovering viruses from environmental samples (Dahling et al., 1974). A second change in the assay

system involved the replacement of Vero cells with RD cells, a line developed from a human rhabdomyosarcoma. It was reported to have a good spectrum for the coxsackie A viruses, many of which had not previously been isolated in cell culture. An extensive study of cell assay systems in our laboratory (Benton and Ward, 1982), indicated that the combined cell cultures used in the survey were sensitive as a group to the vast majority of the known enteric virus types. The entire volume of each sample concentrate was distributed among these cell monolayers which were subsequently observed for CPE for 14 days. If no CPE appeared, a suspension of the cells and medium was passaged to a second container of identical cells and observed for an additional 14 days.

Twenty-five positive control tests were run during the survey using large-volume samples (usually 1,900 liters) of finished water from selected survey sites. About 100 PFU of one of 10 enteric virus types was seeded under blind conditions into samples subsequently processed and assayed according to the study protocol. On 16 of 25 test runs, the seed virus was recovered in the inoculated cell cultures. The seed viruses recovered (and frequency) were as follows: echoviruses 13 (1/1), 24 (1/1) and 27 (1/2); coxsackieviruses A-21 (1/1 with a seed titer of 1,000 PFU) and B-6 (1/2); adenovirus 15 (1/1) and poliovirus 1 (10/13). Viruses used in runs that were unsuccessful in recovering the seed were as follows: echoviruses 18 (1/1) and 27 (1/2), coxsackievirus B-6 (1/2), reovirus 3 (2/2), adenovirus 13 (1/1) and poliovirus 1 (3/13).

The survey was terminated in September, 1978 with 199 samples collected from 54 water supplies in 11 states (Table 2). The initial samples were collected from complete-treatment systems that used contaminated surface water as the source. Later in the study, sites were added primarily in New England where disinfection was the only treatment applied for producing drinking water.

TABLE 2 USEPA SURVEY OF COMMUNITY DRINKING WATER SUPPLIES FOR VIRUSES 1974-1978

Location (No.)	No. Samples Collected	Sample Vol. (1) mean	(range)	Treatment*
Ohio (5)	22	1,069	(988-1,900)	C, D
Illinois (1)	11	1,771	(1,181-1,900)	C
Indiana (3)	51	1,490	(171-1,900)	C
Pennsylvania (16)	22	880	(209-1,900)	C, D
Maryland (1)	2	1,330	(760-1,900)	C
Missouri (5)	46	1,787	(380-1,900)	C
Virginia (1)	8	380	(380-380)	C
West Virginia (6)	21	1,535	(353-1,900)	C
Kentucky (2)	2	1,258	(1,053-1,463)	D
Vermont (9)	9	1,565	(794-1,900)	D
Connecticut (5)	5	568	(414-893)	D

*C = Complete treatment: clarification, filtration and disinfection
D = Disinfection only

No viruses were isolated from any of the samples. This finding, of course, did not prove that these water supplies were free of viruses. The limitations of the methods used in recovering all viruses were well recognized. At very least, the study showed that the control system had prevented virus contamination. At best, the results provided a degree of confidence that treated drinking water was not likely to be a major route of virus transmission.

EXTENDED VIRUS STUDIES OF TREATED MUNICIPAL WATER SUPPLIES

Missouri Study

During the course of the USEPA survey, a municipal water supply was identified that utilized a severely polluted surface source downstream of a major metropolitan area. Primary and secondary-treated sewage was discharged from the city into the river. Fecal coliform counts at the plant intake were higher than at any survey location we had tested with a geometric mean of 6,000/100 ml and a range of 2,300 to 16,000 colonies for the 12 days that samples had been collected. As a component of a more extensive USEPA funded study (O'Connor et al., 1982), the virus quality of the source and finished waters associated with the municipal water treatment plant was determined. Treatment for this supply was provided by an old but well-operated plant that applied full conventional treatment. The intake water and the finished water at this site were tested for viruses periodically over several months. Source and finished water sample volumes were 190 to 380 liters and 1,900 liters, respectively. A subcontractor collected and processed all samples, assayed the source water samples at its laboratory, and shipped the finished water samples to the USEPA laboratory for viral assessment.

The virus recovery process used was the low-pH adsorption, high-pH elution procedure described in the 14th edition of Standard Methods (1976). The virus adsorbent was a yarn-wound fiberglass depth filter (K-27, Carborundum Co.) in sequence with 1.0 and 0.45 µm porosity epoxy-fiberglass-asbestos plate filters (Cox Instruments Co.). In order to process the desired water volume through the adsorbing filters, the source water required clarification. This was accomplished by prefiltration using a 3 to 5 µm porosity cartridge-type depth filter.

In order to enhance virus adsorption, water samples were conditioned by lowering the pH to 3.5 and adding a polyvalent cation (0.5 mM aluminum chloride). For the source water samples, this conditioning was conducted on the prefiltered water. The prefilter was eluted and assayed for viruses separately to determine the proportion of debris-associated viruses. The one liter glycine eluate obtained from each sample was further concentrated by an aluminum hydroxide flocculation procedure. Viruses in the eluate that were adsorbed and entrapped in the floc formed in situ were subsequently eluted in a smaller volume of glycine solution at pH 9.5. The contract laboratory assayed the source sample concentrates on PMK and BGM cells by the plaque technique. Plaques were picked and passaged to fresh cell monolayers to confirm viral etiology. Identification of the isolates was attempted using Lim-Benyesh-Melnick enterovirus sera pools.

A total of 545 viral isolates was made from 216 samples collected over 54 weeks. Fifty-three of these were recovered from the prefilter eluates. Of the 462 (85%) identified isolates, 81% was found to be polioviruses with coxsackieviruses and echoviruses a distant second and third at 13% and 6%, respectively. An average of 2.5 viral plaque-forming units (PFU) per sample was recovered from the source water. Peak recoveries generally occurred in the winter and spring months with averages of 5 to 10/PFU per 380-liter sample. The number of virus isolates was surprisingly low and is believed to reflect the difficulty in recovering viruses from polluted and turbid surface waters rather than representing the extent of the virus challenge to this water treatment system.

The concentrated 1,900-liter finished water samples were shipped on dry ice to the USEPA laboratory in Cincinnati. Upon receipt they were logged in and maintained at -70°C until assayed. The viral assays were performed in the special isolation laboratory and under the conditions used in the virus survey. Samples were assayed by the CPE procedure. The entire concentrate was placed on monolayers of BGM, RD, PMK and HEK cells and observed for 14 days. Five-ml portions of frozen-and-

thawed cells and media were passaged to fresh monolayer of identical cell types and observed for a second 14-day period before determining a sample to be negative for virus.

During the period December, 1976 to March, 1978, a total of 65 weekly samples of finished water was collected and assayed. Eight of these samples were toxic to the cell culture within 48 hours and one inoculated sample produced significant fungal growth. The toxic samples were frozen and thawed and 5 ml passaged to fresh cells without further manifestation of toxicity. The contaminated sample was filtered and passaged without further fungal problems. All other sample concentrates were assayed without difficulty. None of the 65 finished water samples yielded virus isolations in the four cell types in either passage.

Michigan Study

The most recent USEPA virus study of a drinking water supply was stimulated by concern over potential health effects resulting from reaction products of disinfection. The production of trihalomethanes (THMs) by chlorine reacting with organic constituents in source waters had discouraged the practice of pre-disinfection. A current treatment trend is to place the disinfection step later in the process after the THM-precursor chemicals in the water have been reduced by sedimentation. The practice reduces the water-disinfectant contact time and could perhaps compromise the viral quality of the finished water.

A municipal water treatment plant was identified that utilized a contaminated surface water source. Viral analysis of the surface stream prior to the study indicated significant viral contamination. Plant authorities agreed to modify the disinfectant application to a point after filtration to accomodate objectives of a virus study. A 13-month study was initiated at this site in May, 1981 and a complete report of the findings will soon be published (Stetler et al., 1984).

Samples were collected three times per month at four sites within the water treatment plant: intake, post-sedimentation, post-sand filtration and finished. Attempts were made to collect 380-liter samples at the first two sites and 1,900-liter samples at the last two sites. Reduced volumes were obtained from the first two sites on occasions due to clogging of the filters. Samples were collected by the method outlined in the 15th edition of Standard Methods (1981). The virus adsorbent was a single 17.8 cm long borosilicate glass microfiber-epoxy resin filter tube having a nominal porosity of 8.0 µm (Balston, Inc.). Prior to collection of the sample, the water pH was adjusted to 3.5 to enhance virus adsorption to the filter. Virus elution was by a single pass of 1,100 ml of 1% beef extract (pH 9.5) through the filter tube. The eluates were concentated further by the organic flocculation procedure.

Sample concentrates from the source water, post-sedimentation and post-filtration steps in the treatment process were assayed by the plaque technique on BGM cells. In an effort to maximize virus detection in finished water samples, the CPE procedure and four cell culture lines (BGM, HEL-299, L-132 and RD) grown in media containing 5-iododeoxyuridine were used to assay these samples (Benton and Ward, 1982). The entire volumes of the finished water concentrates were examined in this manner. As a test of the efficiency of the recovery procedure, 380-L samples of dechlorinated finished water were seeded with about 100 PFU of either poliovirus 1, coxsackievirus B3 or echovirus 7. The samples were concentrated by the study procedures and assayed by the plaque technique on BGM cells. Virus recoveries from 10 tests conducted over the study period ranged from 14-84% with an overall average recovery of 38% for all three viruses.

Viruses were readily recovered from the source water with peak numbers occurring

in the fall and winter. Of the 365 isolates identified using the Lim-Benyesh-Melnick enterovirus sera pools, 52%, 25% and 23% were determined to be echovirus, coxsackievirus and poliovirus, respectively. The highest numbers were isolated in February (average of two samples was 316 PFU) and the lowest numbers in May (average of three samples was 2 PFU). The 13-month average numbers per 380-liter sample from the intake, post-sedimentation and post-filtration sampling points were 47, 18 and 4, respectively. It was somewhat surprising to us to have isolated viruses from 17 of 36 post-filtration samples. Adequate chlorination downstream of the finished water sampling point clearly was shown to be essential in producing a virologically acceptable water. No viruses were isolated from the 36 finished water samples. It should be stated that the finished water with a pH below 8.0 and a turbidity below 1.0 NTU had been dosed with between 5-7 mg/L of chlorine and stored in a reservoir for at least 16 hours when these samples were taken.

A summary of the studies at the Missouri and Michigan water treatment plants is shown in Table 3. Considerably more viruses were isolated from the Michigan source water. The use of an improved virus recovery method may have been an important factor since both source waters were clearly polluted with sewage. The turbidity of the Missouri source water consistently exceeded 40 NTU and often exceeded 1,000 JTU (the Jackson candle method was used for turbidity >40 NTU). This degree of suspended matter, never seen in the Michigan study, could have significantly interfered with virus recovery. It should be stated that in neither study were coliform indicator levels indicative of virus occurrence. In fact, a negative correlation was observed suggesting that coliform determinations may not predict the presence/absence or degree of viral contamination of a water system. The negative correlation is difficult to interpret. However, the failure of the coliform indicator system for viral contaminants has been a general observation. It is interesting to note that even though enterovirus fecal shedding is known to peak in late summer to early fall, both studies produced the highest number of isolations from the source water during the winter months. This finding probably reflects better survival of the viruses and reduced sewage treatment efficiency of upstream plants at the lower winter temperatures. Regardless of the poor source water at both plant intakes, the finished water consistently met federal coliform and turbidity standards. Post-filtration chlorine residual was relied upon at both plants to produce a microbiologically-acceptable water. Using more rigorous virus detection procedures than were applied to the samples from other sites, viruses were not isolated from the finished water of either plant.

TABLE 3 SUMMARY OF VIRUS STUDIES OF CONVENTIONALLY-TREATED DRINKING WATER DERIVED FROM POLLUTED SURFACE SOURCES AT TREATMENT PLANTS IN TWO STATES

		Source Water			Finished Water		
Study Site	Virus Method*	Assay System**	PFU/380 L Mean	PFU/380 L High	Assay System**	# of Samples	Virus (CPE) per 1900 L
Missouri	TSM,14th	BGM, PMK	2.5	24	BGM, PMK, HEK, RD	65	None
Michigan	TSM,15th	BGM	47	316	BGM, L-132, HEL-199, RD	36	None

*Tentative Standard Method in 14 or 15th edition of Standard Methods

**BGM = African green monkey kidney (Buffalo); PMK = primary African green monkey kidney; HEK = primary human embryonic kidney; RD = human rhabdomyosarcoma; L-132 and HEL-299 = lines of human embryonic lung cells

CONCLUSION

Human enteric viruses clearly occur in drinking water in the U.S. Surveillance

and investigation of waterborne outbreaks and related clinical findings have established a viral etiology for hepatitis and gastroenteritis (Craun, 1981). In the past five years (1978-82) the annual number of reported outbreaks has ranged from 32 to 50 with viruses known to be responsible for around 10% of the outbreaks. An etiological agent was not identified in about half the gastroenteritis outbreaks. A recent review of clinical data suggests that perhaps 23% of these were due to Norwalk-like viruses (Kaplan et al., 1982).

Gamble (1979) accurately pointed out that these outbreaks are typically associated with water supplies that fail to meet accepted sanitary or treatment standards. He also addressed perhaps a more significant waterborne health issue, the low-level transmission theory. Unfortunately, Gamble based his conclusion on the occurrence of waterborne diseases produced by picornaviruses. This group of viruses, although readily isolated from sewage-polluted waters, has never been shown epidemiologically to have waterborne health significance, with the notable exception of hepatitis A. Perhaps a high frequency of asymptomatic infections, a diverse symptomatology and considerable person-to-person transmission has obscured a drinking water transmission role for these agents. In addition, the fecally-shed members of the picornavirus group (enteroviruses) are primarily infectious agents of childhood.

The Norwalk-like agents of gastroenteritis appear to be more likely candidates for low-level drinking water transmission of viral disease. They produce explosive disease in all age groups and appear to be environmentally stable and readily present in municipal sewage. Also, acute gastroenteritis is the second most common illness affecting the U.S. population (USDHEW, 1977). However, two major limitations hinder a full evaluation of the significance of their waterborne transmission. The true occurrence of gastroenteritis is difficult to determine since there is no requirement to report cases to health authorities and medical treatment is often not sought. This greatly hinders an epidemiological approach. A virological approach is also hindered since neither non-human hosts nor in vitro culture systems for studying the agents is available.

Two health-related questions have remained basically unanswered since enteric viruses were first isolated from water systems: (1) are viruses frequently transmitted through treated drinking water and if so, (2) what is the health significance of low-level transmission? The second question cannot be adequately addressed perhaps until sufficient laboratory methods and epidemiological approaches are available for those viruses known to be waterborne, i.e., hepatitis A and the gastroenteritis agents. The first question should continue to be addressed by the study of enteroviruses since, as a group, they probably represent the best surrogate for the known waterborne viral pathogens. Their behavior in the water environment and in treatment systems can be assumed to reasonably mimic hepatitis A and the gastroenteritis viral agents.

This report has addressed the first question in regard to virus occurrence in the U.S. It appears from the very limited data available that viruses probably do not frequently occur in treated drinking water at detectable levels. However, it seems clear from the Michigan study that "adequate" disinfection is essential in preventing virus transmission. In the U.S., 0.5 mg/L of free residual chlorine for 30 minutes at a pH <8.0 has traditionally been assumed to be adequate. The Michigan study was not helpful in evaluating this conventional wisdom. Even though the disinfection step was challenged by naturally-occurring viruses that had penetrated other treatment steps, the chlorine dose and contact time far exceeded the stated acceptable level.

Adequate disinfection may be perceived as an equivocal term in light of recent virus occurrence data. The positive virus findings reported herein from disinfected

water and those that received complete treatment, though controversial in part, are disconcerting. Of perhaps more concern are the findings of Payment (personal communication) in Canada and Keswick et al. (1984) in Mexico of consistent virus isolations from conventionally-treated finished water. Now that rather sensitive virus recovery methods are available, more occurrence studies would seem to be justified. Critical studies of treatment systems that allow virus penetration are needed to determine the cause of treatment failure.

Data generated by occurrence studies with the enterovirus and other culturable enteric viruses will help address the critical health question regarding low-level virus transmission through drinking water. When practical laboratory tools become available to study the true waterborne viral pathogens, these data will help to focus epidemiological studies and other research efforts on water systems that allow virus penetration and on the resulting exposed populations.

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